Assessing Fermentation Quality of Grain Sorghum for Fuel Ethanol Production Using Rapid Visco-Analyzer

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ABSTRACT

The Rapid Visco-Analyzer (RVA) was used to characterize the pasting properties of 68 sorghum grains with a standard 23-min temperature profile. The results showed a strong linear relationship between ethanol yield and final viscosity as well as setback. Ethanol yield increased as final viscosity decreased. A modified RVA procedure (10 min) with an application of α-amylase was developed to simulate the liquefaction step in dry-grind ethanol production. There was a remarkable difference in mashing properties among the sorghum samples with the normal dosage of α-amylase. The sorghum samples which were difficult to liquefy in the mashing step had much higher peak viscosities than the samples that were easily liquefied. The results also showed that the relationship between conversion efficiency and mashing property was significant. Tannins cause high mash viscosities. There was a strong linear relationship between tannin content and final viscosity as well as peak viscosity. The modified RVA procedure is applicable not only for characterization of mashing properties but also for optimization of α-amylase doses for starch liquefaction.

Ethanol production in the United States is undergoing an unprecedented expansion. In 2006, a record 4.9 billion gallons of ethanol was produced from 110 biorefineries located in 19 states across the country. This exceeded the previous year's record by more than 25% (Renewable Fuels Association 2007). Currently, the feedstock for commercial ethanol production is ≈97.5% corn and 2% sorghum. Sorghum is a drought-resistant, low-input cereal grain and interest is growing for using it for bioindustrial applications in the United States (Farrell et al 2006). Researchers and ethanol producers have shown that grain sorghum is a reasonable feedstock (e.g., technically acceptable, fits the infrastructure, and can be economically viable) for ethanol and could make a larger contribution to the nation's fuel ethanol requirements.

In a conventional, dry-grind ethanol process, sorghum is ground and mixed with water to form mash, which is cooked, liquefied, saccharified, and fermented to produce ethanol. Mashes used in industry for production of fuel ethanol usually have a dissolved content of 20–24 g/100 mL of mash and normally a grain-to-water ratio of 1:3 is used (Thomas et al 1995). More recently, fuel alcohol plants have run at alcohol levels as high as 19–20% by volume, with the average being nearer to 16–17% (Kelsall and Lyons 2003). Due to the high solids in mash, viscosity is extremely high during starch gelatinization. In dry-grind processing, thermostable α-amylase enzymes are added as thinning agents to reduce viscosity and partially hydrolyze starch during cooking. Lower mash viscosities improve heat transfer efficiency in the heat exchangers and allow plants to process higher levels of dry solids, which significantly reduces energy in heating mash and cooling the cooked liquefact before fermentation. In addition to high energy consumption, high viscosity also may result in incomplete starch gelatinization and low ethanol yield. Therefore, viscosity of cooked mash could be used as a quality factor for optimizing solids content in the mash, stirring system, and amylase levels.

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The Rapid Visco-Analyzer (RVA) has been used to study starch pasting properties through the classic heat-hold-cool profile (Wrigley et al 1996). Compared with wheat and corn, fewer studies have reported on using RVA to study grain sorghum and its associated products. RVA has been used mostly to investigate the pasting properties of isolated sorghum starches or starches in raw, dehulled, decorticated, flaked, and malted sorghum grains, with solid levels of 8.6–14.0% (w/w) in the slurries (Cruzy Celis et al 1996; Moheno-Pérez et al 1997; Taylor et al 1997; McDonough et al 1998; Beta et al 2000, 2001; Hugo et al 2000; Suhendro et al 2000; Xie and Seib 2000; Beta and Corke 2001a,b; Agu et al 2006). Previous studies reported RVA procedures with temperature profiles of 13, 18, and 22 min. Beta et al (1995) assayed α-amylase in sorghum malts by measuring the reduction in viscosity using a 3-min rapid test. To simulate an industrial mashing process, Goode et al (2005) successfully used RVA as a rheological tool to characterize the effect of the malt/barley adjunct ratio on viscosity and found clear correlations between the level of barley adjunct and the RVA parameters. Agu et al (2006) showed that RVA can be used to assess wheat for production of grain whisky and found that RVA peak and final viscosities were highly correlated with alcohol yield.

We hypothesized that the sorghum cultivars with high starch contents are generally associated with higher RVA peak and final viscosities that result from larger amounts of gelatinized substrates and produce higher ethanol yields than cultivars with low starch contents. Tannins are well known for effects on inhibition of the α-amylase from porcine pancreas (Davis and Hoseney 1979), *Bacillus subtilis* (Reichert et al 1980) and *B. licheniformis* (Wu et al 2007). Measurement of α-amylase activity with RVA described by Approved Method 22-08 (AACC International 2000) is based on the ability of α-amylase to liquefy a starch gel. Thus, we anticipated that tannins in sorghum should be related to RVA parameters to some extent. To date, published literature contains no reports of using RVA to evaluate tannins in grain sorghum.

Many laboratory dry-grind procedures, all of which belong to the batch-cooking system (Kelsall and Lyons 2003), have been developed. For most procedures, fermentation slurry with a first dose of α-amylase was cooked at 90–95°C for 45 or 60 min (Thomas and Ingledew 1990; Ingledew et al 1995, 1999; Thomas et al 1995; Wang et al 1997, 1999; Zhan et al 2003; Wu et al 2006a,b, 2007). After slurry temperature was reduced to 80°C, a second dose of α-amylase was added and liquefaction proceeded for an additional 30 min. In some procedures, all of the required α-amylase was added to the slurry in one step and the slurry was cooked at 80°C for 60 min (Lee et al 2000) or at 85°C (Singh and Graeber 2005) or 90°C (Singh et al 2006) for 90 min before the

subsequent or simultaneous saccharification step. We assume that a modified RVA procedure with optimized temperature, time, solids level, and enzyme dosage could be used to simulate the cooking and liquefaction steps in the dry-grind ethanol process and to quantitatively characterize mashing properties of sorghum grains.

Therefore, the objectives of this study were to characterize the pasting properties of sorghum grains, simulate the cooking step in a laboratory dry-grind process and identify mashing properties, relate the RVA parameters to ethanol fermentation, and optimize α-amylase dosage used for fuel ethanol production.

MATERIALS AND METHODS

Sorghum Cultivars

A population of 68 sorghum genotypes and elite hybrids as described in Wu et al (2007) was obtained from the 2004 winter breeding nursery of NC+ Hybrids (Monsanto subsidiary) in Puerto Rico. The samples were hand-cleaned to remove glumes, debris, and other impurities, packaged in plastic bags, and stored at 4°C until testing.

Sample Preparation

Tannins in sorghum samples with pigmented testas were deactivated using the formaldehyde method of Daiber and Taylor (1982): grain (100 g) of sorghum cultivars was steeped for 6 hr at room temperature in 100 mL of 0.04% (w/v) formaldehyde or distilled water. Grain was then blotted dry and dried at 49°C for 16 hr. The water-steeped samples were used as controls.

Whole kernels (500 g) for some cultivars were decorticated using a tangential abrasive dehulling device (TADD) equipped with an 80-grit abrasive pad. The abrasive pad was shimmed to a minimum distance from the upper plate. The decortication level was controlled to ≈20% (by weight) by adjusting abrasive time.

The original, steeped, and decorticated samples were ground using a mill (Udy Corp., Fort Collins, CO) through a 1.0-mm screen and used for chemical analysis and RVA testing. Samples for ethanol fermentation were ground into fine meals in a grain mill (Magic Mill Products & Appliances, Monsey, NY) set at level III.

RVA Viscosity Measurements

Viscosities of ground sorghum samples during pasting or liquefaction were determined using RVA (model RVA-3D and RVA-4; Newport Scientific, Warriewood, Australia).

TABLE I
Standard 23-min Gelatinization, Pasting, and Setback Profile

Stage	Rapid Visco Analyzer STD2 Profile
Initial temperature	50°C
Initial holding time	1 min
Heating time from 50 to 95°C	7.5 min at heating rate 6°C/min
Holding temperature	95°C for 5 min
Cooling time from 95 to 50°C	7.5 min at cooling rate 6°C/min
Final temperature	50°C
Final holding time	2 min
Total test time	23 min

For model RVA-3D, a 23-min gelatinization, pasting, and setback profile, as described by Approved Method 76-21 (AACC International 2000) was used. The actual profile is outlined in Table I. Ground samples (4.00 g, 14% wb) were dispersed in 25.00 g of distilled water in aluminum canisters. The RVA parameters measured were pasting temperature, peak time, peak viscosity (maximum hot paste viscosity), holding strength (trough at the minimum hot paste viscosity), and final viscosity (viscosity at the end of the test after cooling to 50°C and holding at this temperature). Breakdown was calculated by the difference between peak viscosity and holding strength, and setback was defined as the difference between final viscosity and holding strength.

A 10-min liquefaction test was made in a model RVA-4 as described by Wu et al (2007). The temperature profile was set to maintain a constant block temperature of 95°C for 10 min. An enzyme solution was prepared by diluting 2.30 mL of heat-stable α-amylase (Liquozyme SC DC from Novozymes) to 1 L of distilled water. For most experiments, 1 mL of the enzyme solution containing 2.30 μL of Liquozyme SC DC was added in a canister to liquefy the solids (8.00 g, 14% wb). The enzyme dosage was calculated based on 10 μL of heat-stable α-amylase per 30 g of dry solids in a normal fermentation test. In other experiments, the α-amylase levels in the slurries were multiples (±1) of the normal dosage. The total weight of water and the required enzyme solution was kept constant at 21.00 g (14% wb). Peak viscosity, peak time, and final viscosity were measured.

Before initiating a sample measurement, a plastic paddle was attached to the stirring head of the RVA and zeroed at 160 rpm against air (Goode et al 2005). After a sample was poured into the water, a plastic paddle was inserted into the sample canister, rotated, and jogged up and down by hand for 15–30 sec to remove lumps. For all RVA measurements, the samples were premixed for 10 sec at 960 rpm, whereafter a speed of 160 rpm was applied. Rheological measurement data were recorded at 4-sec intervals and stored by RVA dedicated software.

Analytical Methods

Moisture content was measured using Approved Method 44-15A (AACC International 2000). Total starch content was determined using Megazyme total starch kits according to Approved Method 76-13. Method B involved pretreatment with DMSO at 100°C. Amylose content of starch was analyzed as in Gibson et al (1997) using an amylose-amylopectin assay kit from Megazyme. Tannin content was evaluated using the modified vanillin/HCl assay of Price et al (1978) with catechin as the standard. Ethanol fermentation was as described by Wu et al (2006b).

Experiment Design

A split-plot design was used to investigate the effects of tannins on mashing properties. Three sorghum samples (4193, 4194, and 4202), were selected as whole-plot factors. Four subplot factors were 1) decortication, 2) steeping seed with dilute formaldehyde solution, 3) original untreated seed, and 4) steeping seed with distilled water.

Statistical Analyses

All experiments were performed at least in duplicate. All tabular results quoted are the mean values of repeated experiments.

TABLE II
Rapid Visco Analyzer (RVA) Test Results for Sorghum Samples Using the 23-min Temperature Profile in RVA-3D

Parameter	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temp (°C)
Minimum	911	785	126	1,193	408	7.3	76.7
Maximum	3,213	2,177	1,874	7,308	5,443	10.3	89.6
Average	2,352	1,615	737	5,088	3,473	9.5	87.1
Standard error	36	21	23	35	39	0.067	0.32

Viscosity curves represent measurements of one sample. Analysis of variance (ANOVA), least significant difference (LSD), split-plot design, and linear regression were performed using SAS software (v.9.1, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Pasting Properties of Ground Sorghum Grains

The 23-min standard RVA temperature profile was applied to measure the pasting properties of 68 ground sorghum grains at a solids level of 11.86% (w/w). The numerical data generated by RVA are summarized in Table II. Typical pasting curves selected to represent the 68 sorghum cultivars based on peak viscosities are displayed in Fig. 1. Sample 4222 had the lowest peak viscosity (911 cP) while sample 4224 had the highest peak viscosity (3,213 cP). Moreover, these two samples had the lowest two final viscosities (1,193 and 3,159 cP for samples 4222 and 4224, respectively) and the shortest two peak times (7.9 and 7.3 min for samples 4222 and 4224, respectively). Waxy starches or grains were characterized as taking less time to reach maximum viscosities and having lower end viscosities than the nonwaxy counterparts (Cruzy Celis et al 1996; Hayakawa et al 1997; Sasaki et al 2000; Yanagisawa et al 2006). Therefore, samples 4222 and 4224 were suspected to be waxy cultivars. Amylose analysis verified that amylose contents for samples 4222 and 4224 were 3.0 and 3.2%, respectively. Except for these two waxy cultivars, all of the other 66 sorghum grains gave similar RVA pasting patterns and differed mainly in the magnitude of the viscosities.

The coefficients of determination (R^2) for RVA parameters and total starch, ethanol yield, and conversion efficiency are summarized in Table III. As expected, starch contents in ground sorghum grains had a significant effect on peak viscosity, holding strength, breakdown, final viscosity, and setback. Total starch was highly correlated with final viscosity as well as setback ($R^2 = 0.60$, $P < 0.0001$ for final viscosity and $R^2 = 0.55$, $P < 0.0001$ for setback). The relationships between ethanol yield and RVA parameters such as peak viscosity, holding strength, breakdown, final viscosity, and setback give strong support to the hypothesis that pasting properties of sorghum can be related to ethanol fermentation. There was a strong linear relationship between ethanol yield and final viscosity as well as setback ($R^2 = 0.61$, $P < 0.0001$ for final viscosity and $R^2 = 0.57$, $P < 0.0001$ for setback). However, results of multiple regression showed that the role of starch was dominant ($P < 0.0001$) when combined with final viscosity or setback to predict ethanol yield. Although the effects of pasting properties (peak viscosity, breakdown, final viscosity, and setback) on conversion efficiency were statistically significant ($P < 0.001$), they could only explain <18% of the variation in efficiency.

Laboratory fermentation tests of 68 sorghum cultivars showed that some samples were easily agglomerated, especially at the beginning of the 45-min liquefaction step when flasks with slurries were directly inserted into a 95°C water bath, becoming difficult to liquefy completely. Representative RVA curves of samples with distinctive liquefaction speeds are shown in Fig. 2. As reported by Wu et al (2006b, 2007), the two waxy samples (4222 and 4224), were easily handled and liquefied very quickly. Except for 4222 and 4224, it is clear that there were no differences in peak viscosity, holding strength, and breakdown between the two group samples with different liquefaction characteristics. For example, sample 4211 was easily liquefied but had a higher peak viscosity whereas sample 4182 was easily agglomerated but had a lower peak viscosity. Therefore, viscosities measured using the 23-min temperature profile could not be used to explain the difference in mashing characteristics among the sorghum samples.

Mashing Properties of Ground Sorghum Grains

In all, 25 sorghum cultivars with broad ranges of starch content, peak viscosity, liquefaction speed, and tannin content were selected for a mashing property study using RVA. The 10-min liquefaction test was programmed to simulate the liquefaction process in a

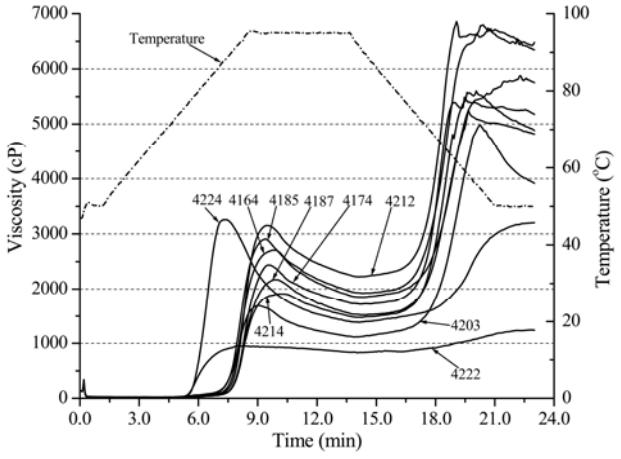

Fig. 1. Pasting curves of nine sorghum samples selected from 68 cultivars and measured using the 23-min temperature profile of the Rapid Visco Analyzer model 3D.

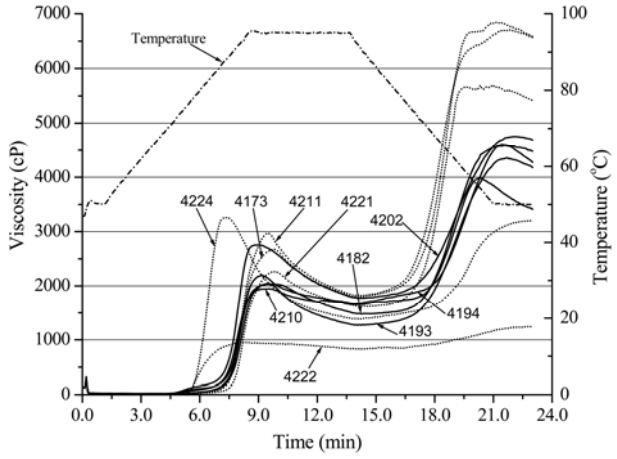

Fig. 2. Pasting curves of 10 sorghum samples selected from 68 cultivars measured using the 23-min temperature profile of Rapid Visco Analyzer model 3D. Samples with a slow liquefaction speed are 4182, 4193, 4194, 4202, and 4210 (solid lines); samples with a quick liquefaction speed are 4173, 4211, 4221, 4222, and 4224 (dotted lines).

TABLE III
Coefficient of Determination (R^2) for Rapid Visco Analyzer (RVA) Parameters and Total Starch, Ethanol Yield, and Conversion Efficiency[a]

Parameter	Peak Viscosity	Holding Strength	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temp
Total Starch	0.44***	0.29***	0.30***	0.60***	0.55***	0.0019ns	0.0060^{ns}
Ethanol Yield	0.47***	0.27***	0.36***	0.61***	0.57***	0.0017ns	0.0043^{ns}
Conversion efficiency	0.16***	0.05^{ns}	0.17***	0.18***	0.18***	0.0003^{ns}	0.0005^{ns}

[a] RVA parameters were measured using the 23-min temperature profile in model RVA-3D; ***, significant at 0.1% level; ns, not significant at 5% level.

laboratory dry-grind procedure. Solids level in the pasting slurry was 23.72% (w/w), similar to the fermentation test. The dosages of α-amylase in the slurries were controlled to be the same if all sound grains had an identical level of endogenous α-amylase. A canister with slurry was placed into the heating sink, just like a flask was inserted into a hot water bath in a fermentation test. Starch in the slurry gelatinized almost immediately when the canisters were put into a block preheated to 95°C (Fig. 3), and viscosity of the slurries increased dramatically. Meanwhile, the heat-stable α-amylase tended to reduce viscosity by liquefying the gelatinized starch. There was a balance between gelatinization and liquefaction that led to peak viscosity. When gelatinization dominated, viscosity increased until reaching the peak value. Viscosity decreased gradually after peaks, with the slurries stirred continuously and the block temperature maintained at a constant of 95°C. Earlier tests showed that, for some samples without tannins, viscosities at a stirring time of 10 min could be reduced to the same levels achieved at 45 min (data not shown), probably because the hydrolytic action by α-amylase is terminated when the average degree of polymerization is ≈10–12 (Aiyer 2005). To save time, the liquefaction procedure ended at 10 min.

The remarkable difference in mashing properties of sorghum grains with a normal dosage of exogenous enzyme is shown in Fig. 3. The difference in mashing properties among sorghum grains suggested that the α-amylase in some samples had been inhibited by some substance, perhaps tannins in those sorghums that contained a pigmented testa (Davis and Hoseney 1979; Reichert et al 1980). This inhibition could retard the excess of α-amylase to the gelatinized starch, increase peak viscosity, elongate peak time, reduce the rate of viscosity breakdown after peak, and increase final viscosity. It is noteworthy that peak time, peak viscosity, and final viscosity were highly correlated with each other ($P < 0.0001$), with R^2 of 0.70 for peak viscosity versus final viscosity, 0.67 for peak viscosity versus peak time, and 0.76 for peak time versus final viscosity.

The 25 samples were divided into three groups (Fig. 3A-C) according to peak viscosities. The samples with a slow liquefaction rate in Fig. 2 were classified into group one (Fig. 3A) and group two (Fig. 3B). All samples with tannins except for 4188 and 4199 belonged to these two groups, suggesting that tannins could be an important factor affecting mashing properties. The samples with a quick liquefaction rate in Fig. 2 were placed in the third group (Fig. 3C). For all 25 samples, regression analysis showed that starch content did not influence peak viscosity ($P = 0.546$). Ethanol yield was not affected by peak viscosity ($P = 0.099$) but was affected by final viscosity ($P = 0.038$). The relationships between conversion efficiency and mashing properties were negatively significant ($R^2 = 0.33$, $P = 0.003$ for peak viscosity and $R^2 = 0.30$, $P = 0.005$ for final viscosity). The two waxy samples (4222 and 4224) are among the top three grains with the lowest peak viscosities (Fig. 3C), which coincides with our observations in the fermentation tests.

The temperature profile in Fig. 3 reflects temperature changes of the block instead of the actual temperature inside the slurries due to heating hysteresis. The block temperature dropped rapidly to ≈85°C after a canister was inserted into the heat sink, but it returned to the 95°C set point in ≈30 sec. Hazelton and Walker (1996) reported that the liquid temperatures did not quite stabilize and were still increasing slightly even at the end of the 3-min test cycle. According to the peak times in Fig. 3 (0.13–1.00 min), the estimated peak temperatures could be 77–92°C based on the results from a 3-min rapid pasting test by Hazelton and Walker (1996).

Effect of Thermostable α-Amylase on Mashing Properties

Based on results shown in Fig. 3, two extreme sorghum samples (4222 and 4194) were selected to investigate the effect of α-amylase on mashing properties. Sample 4222 had the lowest peak viscosity (938 cP) while 4194 had the highest (13,107 cP). The powerful liquefying action of α-amylase, which lowered viscosity sharply even at very low activity (0.1× normal dosage), is shown in Fig. 4. All RVA parameters (peak viscosity, peak time, final

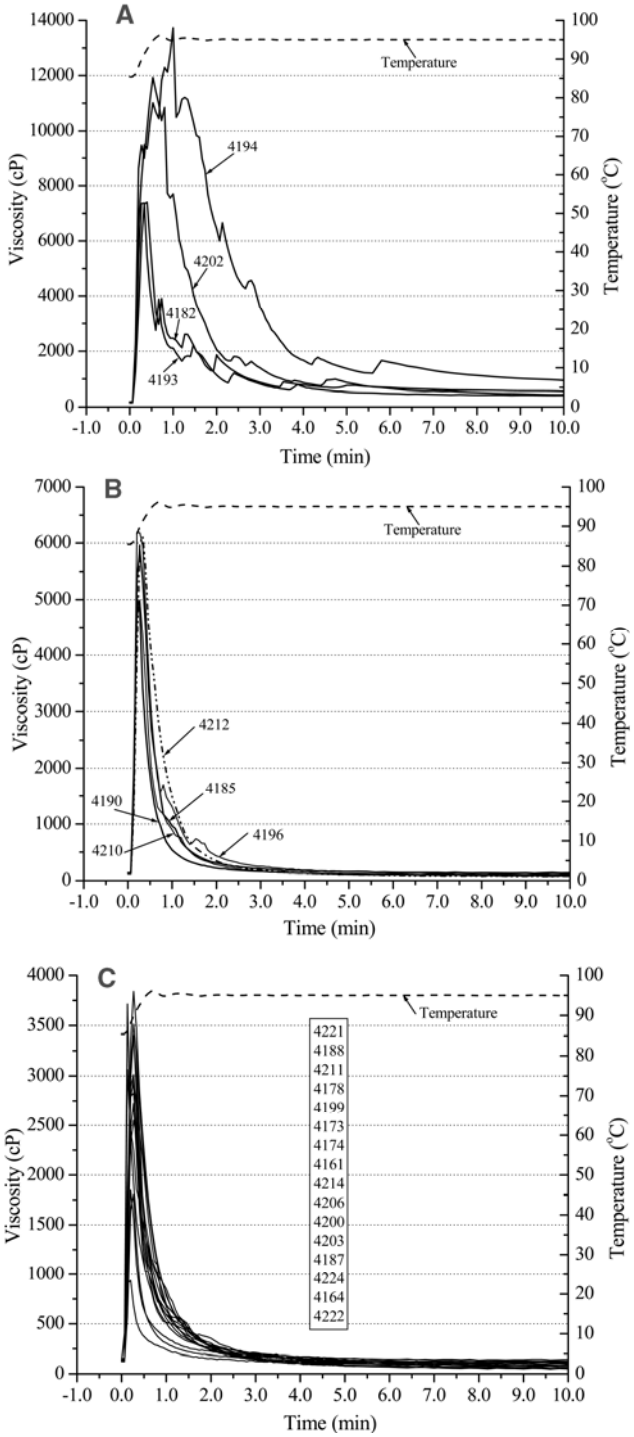

Fig. 3. A, Viscosity curves of four sorghum samples with peak viscosity >7,000 cP, measured using the 10-min temperature profile in Rapid Visco Analyzer model 4 with the normal dosage of α-amylase. **B**, Viscosity curves of five sorghum samples with peak viscosity of 4,000–7,000 cP, measured using the 10-min temperature profile in RVA-4 with the normal dosage of α-amylase. **C,** Viscosity curves of 16 sorghum samples with peak viscosity <4,000 cP, measured using the 10-min temperature profile in RVA-4 with the normal dosage of α-amylase. Sample codes are listed from top to bottom according to peak viscosity, starting with the highest value.

viscosity, rate of viscosity breakdown after peak, and area under curve) decreased remarkably with increasing levels of heat-stable α-amylase in the slurries.

α-Amylase was intensively inhibited in the mashes of sample 4194, which had been reported in a recent study (Wu et al 2007). With increasing enzyme activity, peak time, and final viscosity decreased slightly. However, peak viscosity did not change significantly (P = 0.138), even when the enzyme level was 20× the normal dosage. Sample 4194 was the most difficult to liquefy during mashing in the fermentation tests. Without additional and careful shaking, it was very difficult to disperse and completely liquefy gelled particles.

Effect of Tannins on Mashing Properties

For the nine samples with tannins (Fig. 5), there was a strong linear relationship between tannin content and final viscosity (R^2 = 0.91, $P < 0.0001$) and peak viscosity (R^2 = 0.89, P = 0.0001). In addition, peak time was also highly correlated with tannin content (R^2 = 0.89, P = 0.0001). These results indicate that RVA could be used to quickly predict tannin contents in sorghum grains.

Because tannins are located in the outer layers, pericarp, and testa of sorghum grain (Reichert et al 1980; Hahn and Rooney 1986), mechanical abrasion of the seed coat layers was reported to reduce tannin content (Chibber et al 1978). Steeping seed in dilute formaldehyde solution has been used to decrease tannin content (Daiber and Taylor 1982). Thus, decortication and formaldehyde were applied to remove or inactivate tannins in sorghum grains. Babikir and El Tinay (1993) reported that tannin content can be reduced significantly by steeping whole seed in water at 30°C. For comparison, the water-steeping method was also used in the present study.

Results of the split-plot design are shown in Table IV. Formaldehyde did not react with all tannins in grains, and 23.2–26.3% of total tannins remained after inactivation. Obviously not all outer layers of the seed were removed, but decortication reduced 88.9–96.0% of total tannins when bran removal was ≈20%. For all three sorghum grains, there was no remarkable difference in tannin content between original untreated seed and seed steeped in distilled water. Steeping itself had no effect on tannins.

The inactivation and removal of tannins resulted in significant reduction in peak and final viscosities of sorghum grains (Table IV). After such treatments, the sorghum grains with tannins had peak viscosities similar to nontannin grains shown in Fig. 3. Decorticated samples had slightly larger peak and final viscosities than formaldehyde-steeped samples, possibly due to the higher starch content in decorticated samples (Corredor et al 2006).

Our results showed that samples with extreme high viscosity usually have tannins. However, grains with tannins do not necessarily have problems in liquefaction. For example, tannin content in sorghum 4194 decreased from 39.6 to 10.8 catechin equivalents

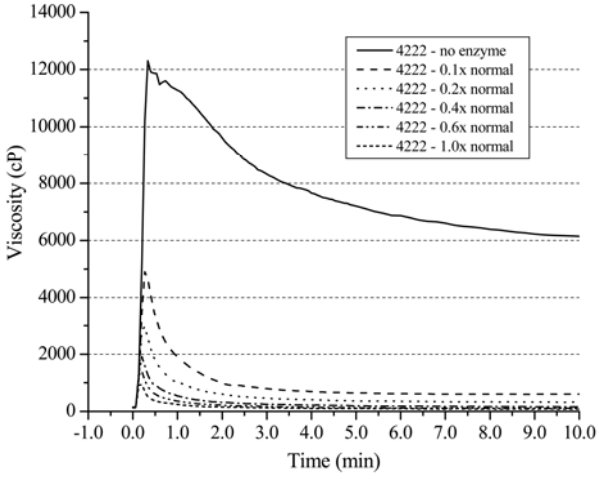

Fig. 4. Viscosity curves of sample 4222 measured using the 10-min temperature profile in the RVA-4 with increased levels of α-amylase in the slurries. Normal dosage of α-amylase was 2.30 μL of Liquozyme SC DC for liquefying the solids (8.00 g, 14% wb) in a canister.

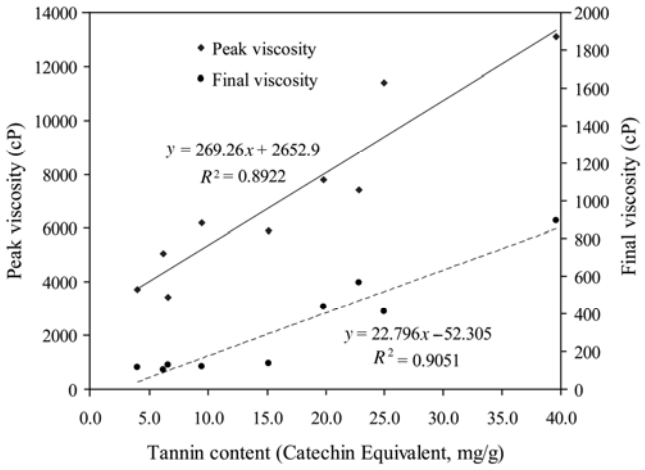

Fig. 5. Relationships between tannin content and peak and final viscosity measured using the 10-min temperature profile with normal dosage of α-amylase.

TABLE IV
Tannin Inactivation by Formaldehyde and Removal by Decortication and Effects of Tannin on Sorghum Mashing Properties Measured Using the 10-min Temperature Profile in RVA-4 with Normal Dosage of α-Amylase

Sample Code	Treatment Description	Tannin Content (CE, mg/g)[a]	Peak Viscosity (cP)[a,b]	Final Viscosity (cP)[a,b]
4193	Decorticated with 19.9% bran removal	0.8 ± 0.1A	2,398 ± 45Aa	183 ± 8Aa
	Steeped in 0.04% formaldehyde for 6 hr	4.6 ± 0.2B	2,296 ± 31Aa	112 ± 3Ab
	Original, untreated	19.8 ± 1.4C	7,781 ± 582Ba	439 ± 21Ba
	Steeped in distilled water for 6 hr	20.4 ± 0.7C	8,331 ± 763Ba	426 ± 27Ba
4194	Decorticated with 20.4% bran removal	4.4 ± 0.1A	4,234 ± 79Aa	134 ± 4Aa
	Steeped in 0.04% formaldehyde for 6 hr	10.8 ± 0.9B	2,742 ± 61Bb	110 ± 3Ab
	Original, untreated	39.6 ± 1.5C	13,107 ± 878Ca	895 ± 88Ba
	Steeped in distilled water for 6 hr	39.0 ± 0.9C	13,721 ± 803Ca	681 ± 54Cb
4202	Decorticated with 22.9% bran removal	2.6 ± 0.4A	4,965 ± 90Aa	110 ± 7Aa
	Steeped in 0.04% formaldehyde for 6 hr	6.1 ± 0.2B	4,413 ± 55Ab	90 ± 7Ab
	Original, untreated	25.0 ± 0.7C	11,401 ± 754Ba	414 ± 30Ba
	Steeped in distilled water for 6 hr	25.0 ± 0.3C	12,405 ± 733Ba	322 ± 17Bb

[a] Mean ± standard deviation of triplicate measurements for tannin contents; means followed by the same uppercase letter for the same sample in the same column are not significantly different ($P < 0.05$). CE, catechin equivalent.

[b] Mean ± standard deviation of duplicate measurements for viscosities; means followed by the same lowercase letter for the first two or the last two treatments applied to the same sample in the same column are not significantly different ($P < 0.05$).

(CE) (mg/g of sample) after treatment by formaldehyde but it was still higher than that of some other original grains with tannins, such as samples 4188, 4190, 4196, and 4199 (Fig. 5). However, samples 4188 and 4199 were detected as having tannins (4.00 and 6.59 CE, mg/g of sample, respectively) but had low peak viscosities (Fig. 3C). Davis and Hoseney (1979) reported that condensed tannins in sorghum contain at least two α-amylase inhibition fractions. Presumably, not all tannin components were responsible for inhibition of α-amylase. It is also possible that the measured tannin values may come from nontannin phenolics that reacted with the reagents but were not really tannins (Dykes and Rooney 2006).

Optimization of α-Amylase Doses for Mashing

In the dry-grind process, the final mash viscosity after liquefaction reflects the degree of starch hydrolysis by α-amylase and thus determines the rate and efficiency of sugar production by amyloglucosidase in the subsequent saccharification or simultaneous fermentation step. It is very important for fuel producers to efficiently reduce mash viscosity to a proper extent using a sufficient quantity of enzymes. It is also valuable for a producer to use the best kind of liquefying enzymes and optimize enzyme dosages which could result in cost savings.

The difference in final viscosities among sorghum grains when the normal dosage of α-amylase was used are shown in Fig. 3. The four samples in the first group (Fig. 3A) had the highest final viscosities (414–895 cP). Three samples had final viscosities of 120–150 cP, two of which were sample 4199 and 4210, both having tannins. All others had final viscosities <120 cP (50–119 cP, average 94 cP), which was taken as a reference value for optimization of α-amylase levels in this study because the samples with final viscosities less than this value had no problems during mashing in the fermentation tests. Succeeding the encouraging results in Fig. 4, all 25 grains were subjected to different levels of α-amylase in the slurries for the purpose of optimization.

Relationships between final viscosities and levels of α-amylase for representative grains are displayed in Fig. 6. Enzyme levels were expressed as multiples of the normal dosage. When the best-fit curves were applied to the data, clear power correlations were found between final viscosity and α-amylase level (R^2 = 0.991, 0.995, 0.995, and 0.981 for samples 4194, 4202, 4212, and 4222, respectively, with $P < 0.0001$). Different grains fitted different power curves, and most grains behaved like samples 4212 and 4222. The optimized enzyme doses to obtain <120 cP of final viscosities varied among grains. The four samples in Fig. 3A required more than twice the normal dosage of α-amylase, whereas nine samples in Fig. 3C needed only 50–80% of the normal level. Final viscosities could be reduced to a value of ≈40–45 cP (Fig. 6), which was the viscosity of a slurry at 60°C before starch gelatinization (data not shown), even with higher enzyme levels.

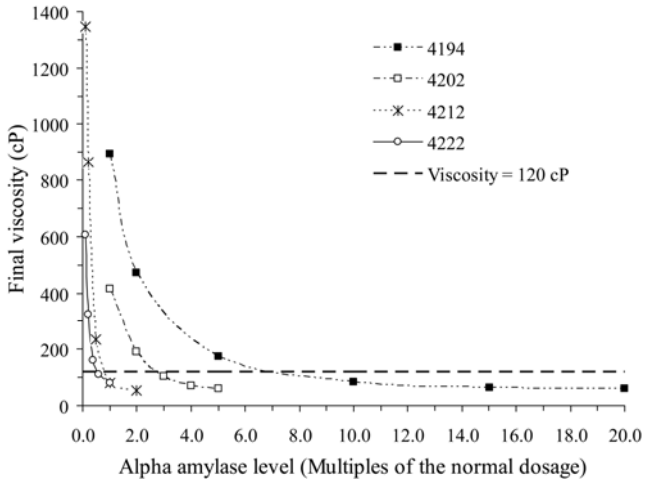

Fig. 6. Effects of α-amylase levels on final viscosities of different sorghum grains measured using the 10-min temperature profile in RVA model-4. Normal dosage of α-amylase contained 2.30 μL of Liquozyme SC DC for liquefying solids (8.00 g, 14% wb) in a canister.

CONCLUSIONS

The feasibility of using RVA as a tool for assessing the quality of grain sorghum to produce fuel ethanol was investigated in this study. For the 23-min gelatinization, pasting, and setback profile, there was a strong linear relationship between ethanol yield and final viscosity as well as setback. From this point, RVA could be used as a tool to predict ethanol yield. Sorghum cultivars with higher peak and final viscosities resulting from larger amounts of gelatinized substrates will produce higher ethanol yields than those with low viscosities. The differences in mashing properties among sorghum grains were enlarged and quantified using the 10-min liquefaction test. There was a remarkable difference in mashing properties among representative grains with normal dosage of α-amylase. It will be very helpful for producers to quickly screen out the grains with abnormally high peak viscosities using RVA. Tannin content was highly correlated to mashing properties. The 10-min RVA procedure could be used as a quick method to predict tannin levels in sorghum grains. For all grains, final viscosities decreased remarkably with increasing levels of heat-stable α-amylase in the slurries. Clear power correlations were found between final viscosities and α-amylase levels. Different grains fitted different power curves, and the optimized enzyme doses to obtain <120 cP of final viscosities varied greatly among grains. These results showed that RVA could be used as a tool to optimize α-amylase doses used for ethanol fuel production. Moreover, RVA could be used for assessment of different commercial enzyme preparations.

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